1941

The Role of Estrogen in Mammary Gland Development and in Lactation.

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The Role of Estrogen in Mammary Gland Development and in Lactation

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Zoology, Physiology and Entomology

By
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A. M., Western Kentucky Teachers College, 1933
1 9 4 1

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Acknowledgment

The writer wishes to express his appreciation for the helpful suggestions and criticisms given by Professor Allan J. Stanley, under whose direction this study was carried out, and for valuable advice and materials given by Professor W. H. Gates, Head of the Department of Zoology, Physiology and Entomology.

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ABstract

Acetone dried powder of anterior lobe of cattle pituitary, containing 50 R. U. of gonadotropic potency per gm., was given to 3 dairy calves over a period of 19 days. The total dosages given were: 3.4 gm. to a 3.5 month old Jersey, 5 gm. to a 6.5 month old Jersey and 7.2 gm. to a 6.5 month old Holstein. Slight mammary gland enlargement was observed in the 6.5 month old Jersey calf. No apparent udder enlargement occurred in the other 2 calves.

A total of 18 mg. of the same pituitary preparation was given to a 34 month old barren Jersey heifer over a period of 17 days. No apparent mammary gland enlargement occurred, but 4 large corpora lutea were found by rectal palpation 22 days after the last injection. Milking was begun 8 days after the first injection. The daily milk volume was 0.41 lb. at the time injection with the synthetic estrogen, diethylstilbestrol dipropionate, was begun. Subsequent treatment of this animal with 10 alternate daily 5 mg. injections of diethylstilbestrol dipropionate was followed by rapid udder enlargement and an increase in milk volume to 4.5 lb. During the 12-day period that followed thetlast injection of this estrogen the daily milk volume increased to 11.5 lb. Lactation continued at a level of 12 lb. per day with a fat content of 7 per cent until the heifer was dried off 8 months after the last injection.

Another experiment with this animal was begun in order to determine the extent of mammary development that could be obtained by injections of diethylstilbestrol dipropionate alone. Alternate daily 5 mg. injections of this hormone were begun 130 days after the end of the first lactation period. The animal came in heat but no mammary gland enlargement occurred. Doses were increased to 10 mg. after the twenty-third injection and continued at this level for 20 days. Two days after the first 10 mg. injection heat reactions ceased and slight udder enlargement followed during the next 10 days. Then the udder increased in size rapidly for a period of 10 days. After this short period of rapid enlargement the udder became flaccid and showed no further increase in size. When 20 mg. injections were begun in 3 alternate daily doses the udder remained flaccid. Milking was begun. During the first 25 days of lactation milk volume increased from 0.41 lb. to 3.7 lb. At this time alternate daily 5 mg. injections of estrogen were begun. The daily volume increased to 8.1 lb. during the first 25-day period of treatment.

Injection of a 22 month old castrate grade Jersey heifer on each alternate day with 5 to 10 mg. doses of diethylstilbestrol dipropionate over a 2-month period produced considerable udder enlargement. Simultaneous injection of 5 to 30 mg. of this estrogen and 1 to 5 mg. of testosterone propionate were then given in alternate daily doses over a period of 83 days. The most rapid increase in size of the udder

occurred when simultaneous injections of 5 mg. of diethylstilbestrol dipropionate and 1 mg. of testosterone propionate were given. Doses exceeding 40 mg. of diethylstilbestrol dipropionate and 10 mg. of testosterone propionate produced little further enlargement. A flaccid condition of the udder followed treatment with 75 mg. of the estrogen alone. At the end of a 9-month period in which 1530 mg. of the estrogen and 536 mg. of the androgen were given, milking was begun. The rate of milk secretion increased in 53 days from 0.41 lb. to 8.6 lb. From this time, daily milk production remained constant for 36 days. A total of 110 mg. of diethylstilbestrol dipropionate was then given in alternate daily 10 mg. injections. Milk production increased to 10 lb. during the first 15 days of this treatment, and then declined to the pre-injection level by the third day after the last injection. A marked increase in production began 36 days after the last injection. Within 18 days the daily yield was 53 per cent greater than it had been at any previous period. After a 21-day period of milk production at a constant level of 13.6 lbs. alternate daily 10 mg. injections of diethylstilbestrol dipropionate were begun. By the sixth day after the first injection the daily production had increased to 15.1 lb. Continued injections were followed by a decline to 13.9 lb. Ten days later the daily milk volume nad increased to 16 lb.

Four pre-pubertal kids were castrated 38 days before injections were begun. Goat 1 was given diethylstilbestrol

dipropionate; goat 2, testosterone propionate; goat 3, three parts of the estrogen to 1 part of the androgen; goat 4, three parts of the estrogen to 1 part of progesterone. Injections were made on each alternate day. Treatment with 1 mg. doses over a period of 46 days induced slight udder growth in goats 1, 3 and 4. The dosage of each hormone was then doubled. The udder of goat 1 became flaccid, and the udder of goat 3 increased in size. After 23 days of treatment with 2 mg. doses, 1.5 mg. injections were begun. Since these injections induced no further udder enlargement over a period of 36 days, injections were stopped.

Diethylstilbestrol dipropionate was given in 0.2 mg. and 1 mg. doses to castrate and intact lactating rats, whose litters had been reduced to 6 at parturition. Injections were given daily from the second to the twentieth day after parturition. The average growth rate of the young was used as the measure of the rate of lactation.

The average growth rate of the young of all treated mothers exceeded that of the controls during the second day of the injection period. After the second day of treatment the young whose mothers were left intact and were given either large or small doses of estrogen, gained weight less rapidly than the sucklings of noncastrate controls. On the contrary the rapid growth rate was continued for 6 days in the young of the castrate mothers which were given 1 mg., and for 12 days in the litters with castrate mothers which were given 0.2 mg. The suppression of lactation was much more pronounced in intact than in castrate animals.

INTRODUCTION

Induction of mammary development with various substances has been demonstrated by several investigators. Allen, Francis, Robertson and Colgate at the University of Missouri, and Doisy, Kountz and Gibson at St. Louis University (1924) reported growth of mammary ducts in mice treated with ovarian extracts. Rather extensive growth of alveoli was noted in the monkey following treatment with a similar preparation by Turner and Allen (1933). Turner and Gomez (1934) induced duct and alveolar development in the guinea pig with estrogen. MacDonald (1936) reported practically no growth of acini when continued injections of estrogen were given to young male rabbits.

According to de Fremery (1936) external application of 10,000 I. U.[1] of estradiol benzoate on the udders of immature goats produced mammary growth. The type or extent of development was not described. Van Heuverswyn, Folley and Gardner (1939) found that a 0.2 mg. dose of diethylstilbestrol produced more pronounced mammary development than a 2 mg. dose in young male mice. Gardner (1941) reported nearly complete inhibition of mammary growth in male mice given weekly injections of 50 gamma of estradiol dipropionate. A similar inhibition of the mammary glands of immature female monkeys and one

1. An international unit (I. U.) is the specific estrus-inducing activity of 0.0001 mg.

male monkey was shown when 1 to 2 mg. of estradiol benzoate were injected weekly for 31 to 36 weeks. Gardner and van Wagenen (1938) found that treatment of an immature castrate female monkey with 136,000 I. U. of hydroestrin benzoate over a period of 21.5 weeks produced complete mammary gland development. It was found that more than 22 weeks were required for complete mammary gland development in young male monkeys given the same treatment. The time factor in breast development of the monkey was emphasized.

The duct and alveolar system of the rabbit was developed with simultaneous injections of estrogen and progesterone by Turner and Frank (1932). Gardner and Hill (1936) reported duct growth in mice treated with 0.1 Corner-Allen unit[2] of progestin daily for 14 days. Selye (1940) observed extensive growth of acini in mature ovariectomized rats when 15 mg. of progesterone were given for 10 days.

Selye, McEuen and Collip (1936 found that daily 0.2 mg. doses of testosterone benzoate for 23 days induced slight acinar tissue development in the female rat 23 days of age. Astwood, Geschickter and Rausch (1937) showed that testosterone stimulates lobule growth in immature male or castrate female rats. By treatment of mature ovariectomized rats with testosterone propionate Reece and Mixner (1939) stimulated

2. A Corner-Allen unit is the minimal amount of an extract which, given in 5 daily doses subcutaneously to a 3 to 4 kg. female rabbit castrated 18 hours post coitum, will produce in the uterus a progestational effect similar to that of the eighth day of pregnancy.

the formation of well developed alveoli.

Gomez and Turner (1937) presented the results of numerous experiments related to the influence of hypophyseal secretions on mammary gland activity in laboratory animals. These results showed that daily implants of rat pituitaries for 20 days from donors previously injected with 100 I. U. of estrogen each day for 10 to 20 days brought about proliferation of the lobule-alveolar system of the mammary glands in normal and castrate male and female guinea pigs from which the hypophysis had been removed. These investigators advanced the theory that growth of the mammary gland is under the direct influence of one or more specific mammogenic hormones of the pituitary which require ovarian hormone stimulation to bring about their secretion. No mammary development was produced in hypophysectomized animals when extracts containing thyrotropin, lactogen and adrenotropin were administered with injections of estrogen and progesterone. Although ovarian hormone injections failed to induce mammary growth after removal of the pituitary, the mucosa of the genital tract proliferated normally. These results were interpreted as further evidence that the hormones of the ovary produce their effect upon the mammary tissue indirectly by way of the pituitary. It was later shown by Gomez and Turner (1938) that development of the duct and lobule-alveolar systems of the mammary glands in castrate rabbits and rats could be accomplished by fresh

or by acetone dried anterior pituitaries from pregnant cattle. Selye (1940) found that 10 mg. doses of crystalline progesterone induced complete mammary development in intact rats but had no effect on the mammary glands of hypophysectomized animals.

The effect of a lactogenic principle upon lactation was reported by Stricker and Grueter (1928) when an increase in milk volume was obtained in normal and low producing cows with injections of anterior pituitary extract. Asdell (1931) brought about lactation in a young goat, which had not been in heat, by treatment with alkaline extract of the anterior lobe of cattle pituitary. With a purified pituitary extract containing lactogen, Catchpole, Lyons and Regan (1934) induced 500 cc. of milk per day in a 16 month old Holstein heifer. Manipulation of the udder in control heifers failed to produce more than a few cc. of milk. Evans (1933) using an alkaline extract of the anterior pituitary produced copious lactation in three virgin goats and in one dry goat. Later Evans (1934) induced lactation in virgin cows and dogs with a similar extract. A maximum of 15 pounds of milk per day was produced in one cow. Gates and Stanley (1938) brought about lactation in a dry and open multiparous deer by intramuscular injection of acetone dried anterior pituitary from cattle. Asimoff and Krouze (1937) gave 6 grams equivalent of crude extract from fresh cattle pituitaries to cows in all stages of pregnancy and lactation.

Some cows failed to show any response while others exhibited as much as a 50 per cent increase in milk volume. Asdell, Brooks, Salisbury and Seidenstein (1936) reported that injections of extracts containing lactogen did not increase production above the maximum provided by the growth of the gland in the lactating goat.

Response to the lactogenic hormone has been reported as negative or very slight in the rat and the mouse by Turner and Shultze (1931). Reece (1939 a) found that lactogen was more effective in initiating lactation in pseudopregnant rats if adrenal cortical extract was injected at the same time. It was suggested that the failure of the lactogenic principle to stimulate milk secretion in the pseudopregnant rat may be due to a deficiency of the adrenal cortex.

Several investigators have shown that estrogens have an inhibitory effect on lactation when given in large doses. De Jongh (1933) found that injections of 250 M. U. of estrogen in oil twice daily into four lactating rats caused death of a majority of the young. Bacsich and Folley (1939) treated intact and ovariectomized rats with 1 mg. of estradiol monobenzoate for 22 days. Although inhibition of lactation occurred in all cases, histological examination of the mammary tissue showed little or no indication of involution. De Fremery (1934) gave 200,000 I. U. of estrogen to a lactating goat and produced a rapid decline in

milk volume. Waterman, Freud and de Jongh (1936) reduced the yield of milk in cattle by rubbing the udder with oil containing 400,000 I. U. of estradiol benzoate. Folley (1936) reported a temporary decline of milk yield in three lactating cows after injection of large doses of estrogen.

The observation that estrogens are excreted in large amounts during the latter part of pregnancy was used by Nelson (1936) in support of the hypothesis that these hormones suppress the release of lactogen from the pituitary. The experimental evidence given by Nelson in favor of this view was obtained chiefly with guinea pigs. This hypothesis does not adequately explain numerous observations in forms other than the guinea pig. In many species such as the mouse, rat, rabbit, goat, cow and man pregnancy may occur shortly after parturition. Lactation may continue in sufficient amount in these animals to raise the young in advanced pregnancy.

Selye (1940) showed that 10 mg. of progesterone given daily to castrate female rats for 20 days caused marked proliferation of acinous tissue of the mammary glands but failed to induce secretion. Histological examination showed that the parenchyma of the mammary gland was extremely compact and the acinar arrangement of the cells was hardly visible because the lumen was collapsed and practically absent in most cases. The mammary gland of castrate female rats receiving 200 gamma of estradiol daily, showed milk in the

acini on the tenth day of treatment. No milk was seen in the alveoli of animals given the same amount of estradiol in combination with 15 mg. of progesterone per day. These observations suggested that progesterone not only fails to stimulate lactation but actually suppresses it, perhaps because it inhibits lactogen formation.

Robson (1937) found that 0.5 mg. of testosterone per day given over a period of three days inhibited lactation in mice although suckling and maternal behavior were not affected.

In a study of the lactogen content of the rat pituitary Reece and Turner (1937) found the greatest concentration of lactogen in glands removed shortly after parturition. During pregnancy the lactogen content of the pituitary did not exceed the amount found in the gland of the estrous female. The pre-pubertal female rat pituitaries contained two to three times more lactogen than was found in the hypophyses from males of the same body weight. Reece (1939) found that the lactogen content of the guinea pig pituitary is lower in diestrum than in estrum. It was also found that the lactogen content of the pituitary increases during pregnancy and the early period of lactation. Reece and Mixner (1939) induced an increase of lactogen in the hypophysis of mature castrate rats by injections of either estrogens or androgens. The lactogen content of the pituitary of various classes of cattle has been reported by Bates, Riddle,

and Lahr (1937). The most striking observation was the high content in embryonic and fetal pituitaries. Reece and Turner (1937) made an extensive study of the lactogen content of cattle pituitaries. It was reported that fetal pituitaries had the lowest lactogen content. The pituitaries from fetuses contained about one-sixth as much lactogen as calf pituitaries. About 85 per cent more lactogen was found in pituitaries from 4 to 10 month old bulls and heifers than in calf glands. The pituitaries of sexually mature heifers contained 58 per cent more lactogen per unit weight than steers of similar age and 70 per cent more than immature heifers. The comparison of the pituitary glands of dairy and beef cattle is especially significant. The hypophyses from lactating and open dairy cattle contained 69 per cent more lactogen per unit weight of anterior lobe tissue than did glands from lactating and open beef cattle; lactating and pregnant dairy cattle 73 per cent more; dry and open dairy cattle 72 per cent more; and dry and pregnant dairy cattle 37 per cent more than the corresponding classes of beef cattle.

Numerous reports exist in the literature indicating that the combined action of estrogen and progestin is necessary for the development of the mammary gland in a manner similar to the normal development during the first half of pregnancy. However, it has been shown that complete development of the lobule-alveolar system in the monkey and guinea pig is induced by injection of estrogen alone. Nelson (1936)

reported that the ovarian hormones which are present in large quantities during pregnancy, are responsible for mammary gland proliferation, but inhibit the secretion and action of the lactogenic hormone. Turner (1939) reported that mammary gland development during pregnancy may be divided into 2 phases. The proliferative phase during the first half of pregnancy is characterized by the rapid growth of the lobules. During the second half of pregnancy the epithelial cells bordering the alveoli of the lobules gradually enlarge and begin to secrete fluid into the lumina of the alveoli. Turner (1933) reported that the rate of estrogen excretion in cattle increases rapidly during the second half of pregnancy.

These observations indicate that a low titre of estrogen induces mammary gland proliferation and a higher titre brings about secretion in the alveolar cells.

In this study experiments were designed in order to produce the titres of estrogen necessary to bring about: first, mammary gland proliferation; second, secretion in the alveolar cells; third, suppression of lactation. Selye, McEuen and Collip (1936) reported slight acinar development in the mammary gland of rats injected with testosterone benzoate. The similarity in mammogenic effect of testosterone together with its close relation in chemical structure to progesterone suggested the use of testosterone propionate in combination with estrogen to bring about

growth of the mammary gland as complete as that found at mid-pregnancy. Acetone dried anterior lobe of cattle pituitary was used in order to increase the amount of secretion of the ovaries to a level that would bring about complete growth of the mammary gland.

Materials and Methods

The cattle pituitaries used in these experiments were collected at the Baton Rouge Abattoir and dried in acetone. After superficial connective tissue had been removed the anterior lobes were dried at 35 degrees centigrade and ground to a powder that would sift through an eighty gauge mesh wire screen. The powder was stored in a refrigerator. The gonadotropic potency of the anterior lobe material was assayed in 21-day female albino rats of the Wistar stock weighing 30 grams or more. Each dose of pituitary powder was weighed on an analytical balance to an accuracy of 0.1 mg., placed in a vial, and suspended in distilled water.

Sesame oil was used as the medium for administration of diethylstilbestrol dipropionate[1], progesterone[2] and testosterone propionate[3].

All injections were subcutaneous. After each treatment in the experimental animal, the site of injection was massaged thoroughly to facilitate absorption. This procedure was adopted after observation that anterior pituitary powder and sesame oil frequently form fluid-filled pockets at the point of injection.

1. Stilbestryl dipropionate contributed by The Lakeside Laboratories, Inc., Milwaukee, Wisconsin
2. Progestone contributed by G. W. Carnrick Co., Newark, New Jersey
3. Oreton contributed by Schering Corporation, Bloomfield, New Jersey, and Perandren contributed by Ciba Pharmaceutical Products, Inc., Summit, New Jersey

The ovaries of a barren Jersey heifer were examined by rectal palpation before and after each series of injections. A diagrammatic sketch of the ovaries showing the number, size, and position of follicles and corpora lutea was made at each examination.

Heat reactions, swelling of the external genitalia and vaginal mucification were used as criteria for hormonal effect on the reproductive system.

A check on the metabolism of diethylstilbestrol dipropionate was made by injection of urine from the treated animals into mature castrate rats according to the Allen-Doisy technique.

The cows used in these experiments were milked twice daily. Tests samples were taken from the morning milking. The official Babcock method was employed for determination of fat content. The solids-not-fat were calculated on a basis of the lactometer reading at 60 degrees Fahrenheit including the percentage of fat as a factor. Total protein was determined by the Kjehldahl method as described in the 1931 edition of the "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists". The addition of 300 cc. of distilled water to the digested sample instead of 200 cc. proved effective in the prevention of bumping during the distillation process. The optical method was used to determine the lactose content. Ash was determined by a gravimetric method.

Turgidity and flaccidity of the udder were used as indicators of the effects of various hormones on the secretory process in the heifers during the periods when milk was not being removed from the developing gland.

Animals used in this study included normal dairy calves, an intact barren Jersey heifer, a castrate virgin grade Jersey heifer, castrate and normal kids, castrate lactating rats and intact lactating rats.

Effect of Cattle Pituitary on the Mammary Glands of Dairy Calves

Acetone dried powder of anterior lobe of cattle pituitary, containing 50 R. U. of gonadotropic potency per gm., was given to 3 dairy calves over a period of 19 days (Table 1). The total dosages given were: 3.4 gm. to a 3.5 month old Jersey, 5 gm. to a 6.5 month old Jersey and 7.2 gm. to a 6.5 month old Holstein. Slight mammary gland enlargement was observed in the 6.5 month old Jersey calf (Fig. 8). No apparent udder enlargement occurred in the other 2 calves (Figs. 6 and 10).

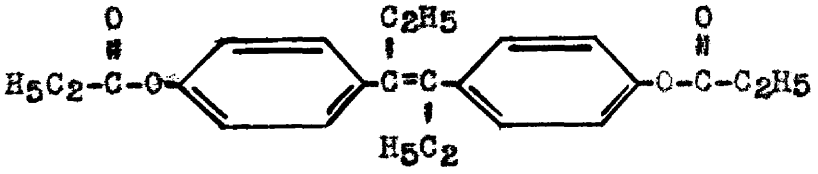
Table 1. The Response of the Mammary Glands of Female Dairy Calves to Pituitary Treatment

Animal	Date	Observations and data
3.5 month old Jersey	July 9, 1939	Udder photographed
	July 10-14	0.2 g. pit.[1] injected daily
	July 16-22	0.4 g. pit. injected on each alternate day
	July 21	5 cc. gonadin[2] injected
	July 23	Udder photographed
	July 23-24	0.4 g. pit. injected daily
	July 25	No udder enlargement occurred
	July 25	Treatment was stopped
6.5 month old Jersey	July 9, 1939	Udder photographed
	July 10-14	0.2 g. pit. injected daily
	July 15	External genitalia swollen
	July 15	Udder slightly enlarged
	July 15	Animal came in heat
	July 15-30	Milky fluid varying from 2 to 15 cc. was expressed from udder daily
	July 16-22	0.4 g. pit. injected on each alternate day
	July 21	5 cc. gonadin injected
	July 23	Udder photographed
	July 23-24	0.4 g. pit. injected daily
	July 25-29	0.8 g. pit. injected on each alternage day
	July 29	No further udder enlargement occurred
	July 29	Treatment was stopped
	July 30	Amount of udder secretion decreased 50 per cent
	July 31	Milking was stopped
6.5 month old Holstein	July 9, 1939	Udder photographed
	July 10-14	0.2 g. pit. injected daily
	July 15-20	0.4 g. pit. injected daily
	July 21	9 cc. gonadin injected
	July 23	Udder photographed
	July 23-24	0.4 g. pit. injected daily
	July 25	1.2 g. pit. injected
	July 27	0.8 g. pit. injected
	July 28	1.0 g. pit. injected
	July 29	No udder enlargement occurred
	July 29	Treatment was stopped

1. Acetone dried powder from anterior lobe of cattle pituitary with 20 R. U. of gonadotropic per gram
2. Pregnant mare serum

Effect of Cattle Pituitary and Diethylstilbestrol Dipropionate on the Mammary Gland of a Barren Jersey Heifer

The next experiment was started in an attempt to determine the effect of the same pituitary preparation on the mammary gland of a 34 month old barren Jersey heifer. The estrous cycles of this animal had been normal since puberty, but frequent mating had failed to bring about conception. A total of 18 grams containing 360 R. U. of gonadotropic potency was administered in alternate daily doses over a period of 18 days (Table 2). This series of injections produced no apparent mammary development; however, a slight milk secretion followed (Fig. 1). Rectal palpation 22 days after the first injection of pituitary material revealed four large corpora lutea. Five days after the last treatment with pituitary powder subcutaneous injection of 5 mg. doses of the synthetic estrogen, diethylstilbestrol dipropionate, was begun. A total of 50 mg. was given in alter-



4,4', dipropionyl alpha, beta diethyl stilbene
(Diethylstilbestrol dipropionate)

nate daily doses. Injection of 15 cc. doses of urine, taken from this animal during this series of injections, did not produce estrus in mature castrate female rats. Twelve days after the first estrogen injection two small corpora lutea

Table 2. The Response of the Mammary Gland of a Barren Jersey Heifer to Pituitary and Estrogen Treatment

Date	Observations and data
Sept. 27, 1939	Normal heat period occurred
Sept. 30	1.5 g. pit.[1] injected
Sept. 31	Rectal palpation showed no corpora lutea
Oct. 3 to Oct. 7	1.5 g. pit. injected on each alternate day
Oct. 7	Udder slightly enlarged
Oct. 7	Milking was begun
Oct. 9 to Oct. 13	3 g. pit. injected on each alternate day
Oct. 16	3 g. pit. injected
Oct. 17	Normal estrus failed to occur
Oct. 21	Rectal palpation showed 4 large corpora lutea
Oct. 22 to Nov. 10	5 mg. est.[2] injected on each alternate day
Oct. 22	Daily milk production 0.13 lb.
Nov. 2	Estrus occurred after a 36-day diestrus
Nov. 3	Rectal palpation showed 2 small corpora lutea
Nov. 6	Udder showed marked hypertrophy
Nov. 6	Udder photographed
Nov. 10	Urine assayed for estrogen with negative results
Nov. 10	Daily milk production 4.5 lb.
Nov. 22	Daily milk production 11.5 lb.
Nov. 22, 1939 to July 6, 1940	12 lb. average for daily milk production
Dec. 6	Rectal palpation showed one small ovarian follicle and no corpora lutea
Dec. 8 to Dec. 26	5 mg. est. injected on each alternate day
Dec. 10	Animal came in heat
Dec. 28	No significant change in milk volume or fat content occurred during the second series of estrogen treatments
July 6, 1940	Milking was stopped
Oct. 7, 1939 to July 6, 1940	Total production: 2880 lb. of milk and 201 lb. of fat
Nov. 11	Udder photographed
Nov. 14	Rectal palpation showed no corpora lutea
Nov. 14 to Dec. 28	5 mg. est. injected on each alternate day
Nov. 18	Animal came in heat

1. Acetone dried powder from anterior lobe of cattle pituitary with 20 R. U. of gonadotropic potency per gram
2. Diethylstilbestrol dipropionate

Table 2. (cont'd)

Date	Observations and data
Nov. 22	Teats became distended
Dec. 30	Udder failed to enlarge
Dec. 30, 1940 to Jan. 21, 1941	10 mg. est. injected on each alternate day
Jan. 1	Heat reactions ceased after 43 days of continuous mating response
Jan. 5	Udder showed slight enlargement
Jan. 14	Rapid udder enlargement began
Jan. 17	Udder turgid
Jan. 20	Udder became flaccid and enlargement ceased
Jan. 26	Animal came in heat
Jan. 26 to Feb. 1	10 mg. est. injected on each alternate day
Jan. 28	Heat reactions ceased
Feb. 3 to Feb. 9	20 mg. est. injected on each alternate day
Feb. 11	Udder remained flaccid and no further enlargement occurred
Feb. 12	Milking was begun
Feb. 13	Daily milk production 0.41 lb.
Mar. 10	Daily milk production 3.7 lb.
Mar. 10 to Mar. 22	5 mg. est. injected on each alternate day
Mar. 25 to Apr. 3	5 mg. est. injected on each fourth day
Apr. 4	Daily milk production 8.1 lb.

could be identified by palpation. During the period of estrogen injection the udder showed marked increase in size (Fig. 12), and the rate of daily milk production increased from 0.13 lb. to 4.5 lb. The daily milk yield had increased to 11.5 lb. 12 days after the last injection. Six days later a maximum of 12 lb. per day was obtained. After a period of 15 days in which the daily production of milk remained rather constant another treatment with estrogen equal in dosage and duration to the first was given. During this series of injections the milk volume did not change. It should be noted that the slight increase in fat content is in contrast to the steady decline that occurred during the first period of estrogen injection (Fig. 1). Until milking was stopped eight months later a daily milk volume of approximately 12 lb. with a fat content of about 7 per cent was maintained.

About 3 months after this animal was dried off alternate daily injections of 5 mg. doses of diethylstilbestrol dipropionate were begun. Heat reactions were displayed on the sixth day of injections with this dosage and the animal continued in heat while this amount of the hormone was given. The dosage of estrogen was increased to 10 mg. 46 days after the first injection. Heat reactions ceased 3 days after the injections with 10 mg. doses were begun. Rapid udder enlargement began 13 days later (Fig. 14). The turgid condition of

the udder which accompanied the 6-day period of rapid enlargement was interpreted as evidence of secretory activity in the mammary gland. As a result of the flaccid condition which followed the estrogen injections were withheld. However, when the animal came in heat 5 days later 10 mg. injections were started notwithstanding the continued flaccid condition of the udder. This procedure was based on the previous observation in this animal and in goats that the most pronounced udder enlargement occurred when a dosage of estrogen slightly above the amount required to induce heat reactions was given. Although heat reactions ceased 2 days after injections were resumed, the udder remained flaccid during a 9-day period in which 10 mg. alternate daily doses were given. A series of 4 injections of 20 mg. alternate daily doses accentuated the flaccid condition of the udder. By palpation of the udder no increase in amount of glandular tissue could be detected. At this point milking was begun. The daily volume of milk increased from 0.41 lb. to 3.7 lb. during the first 25 days of lactation. Alternate daily 5 mg. injections of diethylstilbestrol dipropionate were then given over a period of 13 days.

In order to avoid overdosage subsequent injections were given on each fourth day. At the end of a 25-day period of treatment the daily milk yield had increased to 8.1 lb.

Effect of Diethylstilbestrol Dipropionate and Testosterone Propionate on the Mammary Gland of a Castrate Grade Jersey Heifer

Treatment of a 22 month old castrate grade Jersey heifer with 5 mg. to 10 mg. doses of diethylstilbestrol dipropionate on each alternate day for 51 days produced only slight udder development (Table 3). A fluid, colostral in appearance, was expressed from the front right teat on the twelfth day of the experiment, but milking was discontinued in order to avoid any effect that nervous stimulation might have on the progress of mammary development. Slight swelling of the external genitalia was noted on the thirty-seventh day after the first estrogen injection. The estrogen treatment was followed with simultaneous injection of various ratios of diethylstilbestrol dipropionate and testosterone propionate. Treatment with 5 mg. of the estrogen (diethylstilbestrol dipropionate) and 1 mg. of the androgen (testosterone propionate) was followed by pronounced udder enlargement. After one month of administration of these quantities mammary growth was much less marked. In an effort to augment this slow rate of development various increases in dosage and changes in proportions of the two hormones were made. Simultaneous injection of 40 mg. of diethylstilbestrol dipropionate and 5 mg. of testosterone propionate were found to be the most effective in bringing about further growth of the udder. After 65 days of this treatment mammary gland enlargement was no longer apparent. During the next 48 days increased doses of the

Table 3. The Response of the Mammary Gland of a Castrate Grade Jersey Heifer to Estrogen and Androgen Treatment over a Prolonged Period

Date	Observations and data
June 7, 1938	Born by a grade dam; sire unknown
Mar. 16, 1939	Heifer castrated
Nov. 11, 1939 to Nov. 25	10 mg. est.[1] injected on each alternate day
Nov. 20	Animal weighed 475 pounds
Nov. 21	Urine assayed for estrogen with negative results
Nov. 25	Udder enlarged slightly
Nov. 27	5 mg. est. injected on each alternate day
Dec. 11, 1939 to Jan. 6, 1940	10 mg. est injected on each alternate day
Dec. 22	External genitalia swollen
Dec. 24	Udder enlargement more pronounced
Jan. 8 to Jan. 18	5 mg. est plus 5 mg. and.[2] injected on each alternate day
Jan. 10	Animal came in heat; mounted other other cattle in pasture
Jan. 12	Udder enlargement continued
Jan. 20	5 mg. est. injected
Jan. 23	5 mg. est plus 5 mg. and. injected
Jan. 25	5 mg. est. injected
Jan. 27	5 mg. est. plus 5 mg. and injected
Jan. 29	5 mg. est. injected
Jan. 31 to Feb. 28	5 mg. est. plus 1 mg. and. injected on each alternate day
Feb. 4	Marked udder enlargement occurred
Mar. 1	Rate of udder enlargement very slow
Mar. 1	10 mg. est. plus 1 mg. and. injected
Mar. 3 to Mar. 11	5 mg. est. plus 1 mg. and. injected on each alternate day
Mar. 13 to Mar. 23	10 mg. est. plus 2 mg. and. injected on each fourth day
Mar. 25 to Apr. 3	10 mg. est. plus 1 mg. and. injected on each alternate day
Apr. 5	15 mg. est. plus 2 mg. and. injected
Apr. 7	20 mg. est. plus 2 mg. and. injected
Apr. 11	30 mg. est. plus 3 mg. and. injected
Apr. 13	35 mg. est. plus 5 mg. and. injected
Apr. 15 to Apr. 26	40 mg. est. plus 5 mg. and. injected on each alternate day
Apr. 20	Rate of udder enlargement increased
Apr. 30 to June 19	40 mg. est. plus 5 mg. and. injected on each fourth day

1. Diethylstilbestrol dipropionate
2. Testosterone propionate

Table 3. (cont'd)

Date	Observations and data
June 24	45 mg. est. plus 5 mg. and. injected
June 27	50 mg. est. plus 5 mg. and. injected
June 30 to July 3	55 mg. est. plus 5 mg. and. injected on each fourth day
July 6 to July 12	55 mg. est. plus 10 mg. and. injected on each fourth day
July 7	Animal came in heat. Mounted other animals in pasture
July 15 to July 21	55 mg. est. plus 15 mg. and. injected on each fourth day
July 22	Udder flaccid
July 24 to July 27	55 mg. est plus 5 mg. and. injected on each fourth day
July 26	Udder turgid
July 27	External genitalia swollen
July 30	55 mg. estrogen injected
Aug. 1	Animal went out of heat
Aug. 2	65 mg. estrogen injected
Aug. 5	75 mg. estrogen injected
Aug. 6	Udder flaccid
Aug. 8	65 mg. est. plus 10 mg. and. injected
Aug. 11	40 mg. est. plus 10 mg. and. injected
Aug. 13	Milking was begun
Aug. 14 to Oct. 6	Daily milk volume increased from 0.41 lb. to 8.6 lb.
Oct. 7 to Nov. 9	8 lb. average for daily production
Nov. 9 to Nov. 29	10 mg. est. injected on each alternate day
Nov. 24	10 lb. milk yield
Dec. 1	Milk production declined to the pre-injection level
Dec. 2, 1940 to Jan 5, 1941	8.1 lb. average for daily production
Jan. 4	Animal weighed 665 pounds
Jan. 24	Milk volume 53 per cent above pre-injection peak of production
Jan. 24 to Feb. 15	13.6 lb. average for daily production
Feb. 15 to Feb. 25	10 mg. est. injected on each alternate day
Feb. 18	15.1 lb. milk yield
Feb. 19 to Feb. 22	Daily milk yield declined to 13.9 lb.
Feb. 23 to Mar. 4	13.9 lb. average for daily production
Mar. 7 to Mar. 28	15 lb. average for daily production
Aug. 13, 1940 to Mar. 28, 1941	Total production: 2070 lb. milk and 104 lb. fat.

estrogen and the androgen or the estrogen alone failed to induce further growth. These large doses caused the udder to become flaccid. At this time milking was begun.

The first milk had the physical appearance and approximate chemical composition of colostrum. The establishment of complete lactation proceeded at a rather slow rate (Fig. 2 and Appendix). Fifty-three days after milking was begun an 8.6 lb. production peak was reached. An average of 8 lb. per day was produced during the next 36 days. At this point in the experiment 10 mg. injections of diethylstilbestrol dipropionate were begun. These doses were given on each alternate day. Milk volume increased consistently. On the sixteenth day the production of 10 lb. exceeded the pre-treatment peak by 16 per cent (Fig. 2 and Appendix). Continued estrogen injection was accompanied by a gradual decline in milk volume. A total of 110 mg. of the estrogen were given. Milk production returned to about the same level as that of the original plateau and remained consistent for 36 days following the last injection of the hormone. At this time a marked increase in the rate of lactation began. Within eighteen days the daily production of 12.2 lb. was 53 per cent greater than it had been at any period prior to the estrogen injections. Daily production continued at the level of 13. 6 lb. for 21 days. Another series of alternate daily injections of diethylstilbestrol dipropionate in 10 mg. doses was begun at this time. The daily production increased to

15.1 lb. by the sixth day after the first injection and then declined gradually. A pronounced swelling of the genitalia 12 days after the first injection suggested a high titre of estrogen. Injections were then stopped. A daily production level of 13.9 lb. was established during the 10-day period following the last injection. Then the milk volume increased to 16 lb. per day and has remained constant up to the time of this writing 22 days later.

Analyses of duplicate samples of milk, taken on 3 successive days about 3 months after the beginning of lactation, gave the following averages for the 3-day period: protein, 3.8 per cent; lactose 5 per cent; ash, 0.74 per cent; fat, 5.3 per cent; solids-not-fat, 9.06 per cent. The percentages of these constituents indicate that the chemical composition of the milk was normal at this time.

Effect of Diethylstilbestrol Dipropionate, Testosterone Propionate and Progesterone on the Mammary Glands of Goats

The failure of anterior pituitary injections to induce mammary gland development in dairy calves raised the question of age as a possible determining factor in the milk-producing system of pre-pubertal animals. The pronounced mammary gland development which followed the injections of testosterone propionate in the castrate heifer made it desirable to compare the effect of this hormone to similar injections of progesterone. Since the response of the mammary gland to testosterone propionate was obtained when estrogen was being given simultaneously, it seemed necessary to test the mammogenic property of these two hormones given alone in separate animals.

Since sufficient numbers of cattle were not available for the study of these problems a series of experiments with goats were set up. Kids that had never been in heat were selected for these experiments in order to test the effect of estrogen and progesterone on the mammary glands before they had been affected by the increased activity of the ovaries at puberty. Since there were 2 sets of twins in this group of animals better controlled experiments were made possible.

Six common kids not of a milking breed were used. Five of these animals were castrated and one was left intact as a control. Treatment was begun 38 days after castration.

Injections were given in alternate daily doses to the first four kids and the fifth served as a castrate control. The first and second pairs of goats were twins. At the beginning of treatment the first experimental animal received 1 mg. of diethylstilbestrol dipropionate; the second, 1 mg. of testosterone propionate; the third, 0.75 mg. of diethylstilbestrol dipropionate and 0.25 mg. of testosterone propionate; and the fourth, 0.75 mg. of the estrogen plus 0.25 mg. of progesterone (Table 4). The udders of all the animals were manipulated daily. On the eighth day of treatment, goats 1, 3 and 4 showed evidence of vaginal mucification by abundant mucous secretion adhering to the vulva. Nipple development was noted ten days after the first injection in all goats receiving treatment. Lengthening of the teats was most pronounced in the kid treated with testosterone propionate. Slight udder development was observed in goats 1, 2 and 4 sixteen days after treatment was begun. Goat 1 came in heat 28 days after the first injection.

Since 46 days of treatment with 1 mg. injections had induced only slight udder growth the amount of each hormone was doubled in subsequent injections.

Four days after the beginning of treatment with 2 mg. doses heat reactions occurred in goats 2, 3 and 4 and continued for several days. Heat reactions ceased in all of the treated animals by the twelfth day after the 2 mg. injections were begun. The udder of the kid injected with diethylstilbes-

Table 4. The Response of Castrate Female Kidstto Estrogen, Androgen and Progesterone Treatment over a Prolonged Period

Goat No.	Date	Observations and data
1	Nov. 14-Dec. 28	1 mg. est.[1] injected on each alternate day
	Nov. 22	Vaginal mucification
	Nov. 30	Slight udder development
	Dec. 12	Animal came in h at
	Dec. 16	Udder turgid
	Dec. 30-Jan. 21	2 mg. est. injected on each alternate day
	Jan. 11	Heat reactions ceased
	Jan. 11	Udder flaccid
	Jan. 28-Mar. 5	1.5 mg. est. injected on each alternate day
	Mar. 7	No further udder enlargement occurred
2	Nov. 14-Dec. 28	1 mg. and.[2] injected on each alternate day
	Nov. 24	Teats lengthened
	Dec. 30-Jan. 21	2 mg. and. injected on each alternate day
	Jan. 3	Animal came in heat
	Jan. 9	Heat reactions ceased
	Jan. 28-Mar. 5	1.5 mg. and. injected on each alternate day
	Mar. 7	No udder enlargement occurred
3	Nov. 14-Dec. 28	0.75 mg. est. and 0.25 mg. and. injected on each alternate day
	Nov. 22	Vaginal mucification
	Nov. 30	Slight udder development
	Dec. 30-Jan. 21	1.5 mg. est. and 0.5 mg. and. injected on each alternate day
	Jan. 3	Animal came in heat
	Jan. 9	Heat reactions ceased
	Jan. 13	Udder development enhanced
	Dec. 28-Mar. 5	1.125 mg. est. and 0.375 mg. and. injected on each alternate day
	Mar. 7	No further udder enlargement occurred
4	Nov. 14-Dec. 28	0.75 mg. est. and 0.25 mg. progesterone injected on each alternate day
	Nov. 22	Vaginal mucification
	Nov. 30	Slight udder development
	Dec. 30-Jan. 21	1.5 mg. est. and 0.5 mg. progesterone injected on each alternate day
	Jan. 3	Animal came in heat
	Jan. 9	Heat reactions ceased
	Dec. 28-Mar. 5	1.125 mg. est. and 0.375 mg. progesterone injected on each alternate day
	Mar. 7	No further udder enlargement occurred

1. Diethylstilbestrol dipropionate
2. Testosterone propionate

trol dipropionate became flaccid. The udder of the kid treated with the estrogen and testosterone propionate showed an increase in rate of enlargement while no effect could be detected in the twin receiving progesterone with the estrogen. No udder enlargement occurred in the animal treated with testosterone propionate alone. After 23 days of treatment with 2 mg. doses injections were discontinued. Goats 1 and 4 came in heat on the fourth day and sixth day, respectively, after the cessation of injections.

Injections of 1.5 mg. doses were begun in the goats in an attempt to produce a hormone titre slightly higher than the level necessary to produce estrus. Goat 4 went out of heat 6 days after these injections were begun. Goat 1 continued in heat. After 36 days of these injections no further enlargement of the mammary glands occurred. Injections were then discontinued. Goat 1 came in heat 4 days after the last injections. This animal went out of heat 2 days later.

Effect of Diethylstilbestrol Dipropionate on Lactation in Rats

The increase in rate of milk secretion which followed the first few 10 mg. doses of a series of injections of diethylstilbestrol dipropionate in the castrate heifer is not in agreement with the theory that estrogen inhibits lactation which was proposed by Nelson (1936). A gradual decline in milk production followed continued injections of 10 mg. doses of estrogen. These observations suggested that the inhibitory effect of estrogen might be caused entirely by high titres. Accordingly, a series of experiments were begun with lactating rats in which varied amounts of this hormone were given. A total of 26 multiparous females averaging 240 gm. in body weight were divided into 6 groups. Six served as normal controls; four intact and four castrate animals were given daily injections of 0.2 mg. of diethylstilbestrol dipropionate from the second to the twentieth day of lactation; four noncastrate and four castrate females were treated in the same way with 1 mg. injections of this hormone; four castrate females were used as controls. In all cases the litters were reduced to six at birth. All castrations were performed on the first day after parturition. The growth rates of the sucklings were used as indices of lactation. Litters were discarded when one or more of the young died. Mothers were autopsied at the twenty-first day of lactation or at the time when the last suckling in the

litter died, and the weights of the pituitary, adrenals, ovaries and uterus were recorded.

Average growth rate of the young of all groups of treated mothers exceeded that of the young of untreated mothers during the second day of the injection period (Figs. 3 and 4 and Tables 5 and 6). After the second day of treatment, the young whose mothers were left intact and were given either large or small doses of diethylstilbestrol dipropionate, gained weight less rapidly than the sucklings of the non-castrate controls. On the contrary the rapid growth rate was continued for a longer period in the young of the castrate animals which were treated with this hormone. The weight of sucklings of mothers which were given 1 mg. doses exceeded the weight of the young of castrate control animals through the sixth day. The litters of castrate animals treated with 0.2 mg. doses maintained an average weight above that of the controls through the fourteenth day. It should be pointed out that the inhibitory effect of this estrogen was much more pronounced in intact than in castrate animals. Either large or small doses of the hormone brought about almost complete suppression of lactation by the eighth day of treatment in noncastrate animals. Doses of 1 mg. produced complete inhibition in 2 of the castrate mothers, but this dosage failed to suppress lactation completely in 2 other castrate animals. Doses of 0.2 mg. produced little or no inhibitory effect in 3 castrate mothers, although the young

Table 5. The Average Growth Rate of Sucklings of Noncastrate Rats Injected with Diethylstilbestrol Dipropionate

Days after birth	Average weight of young in gm.								
	No. of mothers given 0.2 mg. estrogen (daily)				No. of mothers given 1 mg. estrogen (daily)				No. controls
	1	1	1	1	1	1	1	1	6
2[1]	6.0	6.4	58.8	6.9	5.2	7.1	6.7	5.2	6.3
3	7.5	7.6	6.8	8.1	6.8	8.1	7.9	6.0	7.1
4	8.5	8.8	7.4	9.0	7.8	8.9	9.6	6.9	8.3
5	9.6	9.7	7.9	9.9	10.2	9.2	10.6	7.4	9.6
6	10.8	10.5	7.8	11.2	11.2	9.6	11.6	7.8	10.8
7	11.3	11.3	7.8	11.9	11.8	9.5	12.4	7.9	12.0
8	11.9	11.6	2 d.	12.4	12.3	9.3	12.9	8.1	13.2
9	12.2	11.8		12.7	12.9	3 d.	13.3	8.4	14.3
10	12.1	11.8	1 d.	13.3	12.7		14.0	8.4	15.4
11	12.0	11.7	3 d.	14.0	14.0		14.5	7.9	16.7
12	11.9	10.6		14.1	13.5		14.3	1 d.	18.2
13	10.9	9.5		14.4	12.3		15.0	5 d.	19.1
14	1 d.	6 d.		14.6	12.2		15.4		20.3
15				15.2	2 d.	1 d.	15.8		21.5
16				15.7	2 d.		15.9		23.1
17	2 d.			15.6		2 d.	16.2		23.8
18	1 d.			15.6			16.2		25.1
19				14.7	1 d.		16.3		26.8
20[2]				14.3			16.6		29.1
21				1 d.			17.0		31.0

1. First injection 2. Last injection d. Death of young

Table 6. The Average Growth Rate of Suckling Rats of Castrate Mothers Injected with Diethylstilbestrol Dipropionate

Days after birth	Average weight of young in gm.					
	No. of mothers given 0.2 mg. estrogen(daily)		No. of mothers given 1 mg. estrogen(daily)			No. controls
	3	1	2	1	1	4
2[1]	6.7	5.5	5.7	6.2	5.0	6.0
3	8.1	6.5	6.8	7.3	5.6	7.0
4	9.4	7.8	8.4	8.1	6.9	8.0
5	10.8	8.3	9.6	9.2	7.5	9.0
6	12.0	9.3	10.6	10.2	8.6	9.7
7	13.0	8.8	11.8	10.6	9.4	11.0
8	14.1	8.6	12.6	10.3	9.6	11.8
9	15.2	8.9	13.3	11.2	10.0	13.3
10	16.1	1 d.	13.8	11.9	9.7	14.4
11	17.1	1 d.	14.6	12.6	9.7	15.7
12	18.1		14.8	13.3	1 d.	16.8
13	19.1		15.8	12.7		18.0
14	19.9	1 d.	16.4	1 d.	3 d.	19.2
15	20.5		16.3		2 d.	20.9
16	21.3		17.4			22.4
17	22.0	1 d.	18.1			23.4
18	22.6	2 d.	17.9			25.1
19	24.1		18.9			26.3
20[2]	26.0		19.7			28.8
21	28.2		20.6			30.6

1. First injection 2. Last injection d. Death of young

of a fourth animal given this dosage began to lose weight on the fifth day of treatment.

Table 7 shows the effect of the injection of this hormone on the weight of the endocrine glands studied in these experiments. It should be pointed out that the endocrine glands of those animals which were injected show a pronounced increase in weight when compared with the glands in the control mothers. Examination of the results showed no effect on gland weight that could be attributed to size of dosage or to castration.

It was found that all treated animals lost weight during the period of injections. The average loss of weight in the intact rats was about double that of the castrates. On the other hand, it should be pointed out that castrate control animals lost weight while the intact untreated animals gained weight during the 21 days of lactation.

The premature opening of the vagina of the sucklings of injected animals suggests that large amounts of diethylstilbestrol dipropionate were secreted with the milk. The vagina was open 12 days after birth in most of the treated litters.

Table 7. The Effect of Diethylstilbestrol Dipropionate on Lactating Female Rats

Description	No. of inj.	Amt. est.[2] (mg.)	Init. wt. (gm.)	Fnl. wt. (gm.)	Ovar. wt. (mg.)	Adr. wt. (mg.)	Pit. wt. (mg.)	Uterine wt. (gm.)
Castrate	19[1]	0.2	234	221		89.2	21.6	1.1718*
Castrate	19	0.2	244	202		71.2	31.7	.4956
Castrate	16	0.2	249	185		84.6	17.4	.6977
Castrate	19	0.2	236	212		80.8	20.5	.5532
Castrate	14	1	275	230		96.8	20.6	1.0317*
Castrate	19	1	222	214		68.5	20.5	1.0160
Castrate	13	1	238	194		108.2	28.2	.8140
Castrate	19	1	272	244		87.3	25.4	.7509
Castrate			160	187		40.0	91.1	.0750
Castrate			246	232		73.8	13.7	.2366
Castrate			230	217		64.3	10.5	.1708
Castrate			245	234		54.5	11.5	.1688
Intact	18	0.2	255	248	65.8	88.8	21.8	1.0828
Intact	12	0.2	223	205	89.6	89.2	20.4	1.1545*
Intact	9	0.2	254	178	126.5	108.0	15.0	.9613
Intact	18	0.2	272	225	93.2	107.7	20.6	.8240
Intact	19	1	230	221	84.5	92.2	16.1	1.0122
Intact	15	1	298	261	91.1	134.4	23.3	.7653
Intact	19	1	249	214	92.6	102.0	38.6	.7364
Intact	11	1	294	died				
Intact			206	211	49.1	77.0	7.3	.1610
Intact			219	243	46.9	59.5	8.0	.1518
Intact			261	256	64.0	56.8	16.0	.4914
Intact			259	266	50.9	63.8	10.8	.2687
Intact			240	248	42.5	48.6	13.2	.2186
Intact			232	236	51.1	76.5	3.0	.3015

* Weight of uterus and vagina.
1. All animals were autopsied 1 day after the last injection.
2. Diethylstilbestrol dipropionate.

Discussion

As a result of a great number of investigations it has been shown that the ovarian hormones and possibly one or more mammary growth factors from the pituitary are primarily responsible for the development of the mammary system. Many papers have been presented as evidence that a lactogenic principle secreted by the hypophysis is essential for initiation and maintenance of milk secretion. Gaunt and Tobin (1936) showed that the life-sustaining dosage of the adrenal cortical hormone was insufficient to maintain lactation in rats. By doubling the life-sustaining dosage lactation was sufficient to rear all litters. Most of the literature agrees with the observation of Grimmer (1918) that thyroidectomy materially reduces the level of milk secretion, although lactation will proceed in the absence of the thyroid.

In consideration of the number of pituitary hormones related either directly or indirectly to mammary development and lactation it was deemed expedient to utilize the entire anterior lobe of the pituitary in order to attain the amount of each hormone most conducive to mammary function. Our experiments with dairy calves of different ages and breeds suggest that there is a difference in responsiveness of the milk-producing system due to age. Slight udder enlargement occurred in a 6.5 month old Jersey calf while no apparent response was shown in a 3.5 month old Jersey calf when these

animals were injected with acetone dried anterior lobe of cattle pituitary.

Although treatment of the noncastrate barren Jersey heifer with anterior lobe powder produced no apparent mammary growth, 4 large corpora lutea were found 22 days after the initial injection. The presence of these corpora lutea suggests that the ovaries may have passed through the peak of the estrogen-producing phase in a period too short to induce a noticeable effect upon the udder. Dodds, Goldberg, Lawson and Robinson (1938) found that 0.5 gamma of diethylstilbestrol kept rats in estrus from 4 to 5 days whereas 10 gamma of diethylstilbestrol dipropionate, which is one half as active biologically per unit weight, maintained estrus for 50 days. The prolonged effect of this estrogen as reported for the rat suggests its efficacy in the stimulation of mammary gland development in animals with long reproductive cycles. The small amount of this hormone required to accomplish mammary proliferation in the barren Jersey heifer was probably due to the additive function of the hormones from the animal's own ovaries. The presence of 4 large corpora lutea at the beginning of estrogen injections indicates a high rate of secretion of progesterone. The rapid increase in milk volume following the cessation of estrogen injections suggests that the titre following this dosage was not sufficiently high to effect suppression of the secretory process.

It should be pointed out in light of later results with the noncastrate heifer that the decrease in fat content accompanying the first series of estrogen injections did not occur when this hormone was given after maximum lactation had been established. This observation was reaffirmed by estrogen treatment of the lactating castrate heifer. Doses twice as large as the 5 mg. injections given to the noncastrate heifer produced no significant change in the fat content.

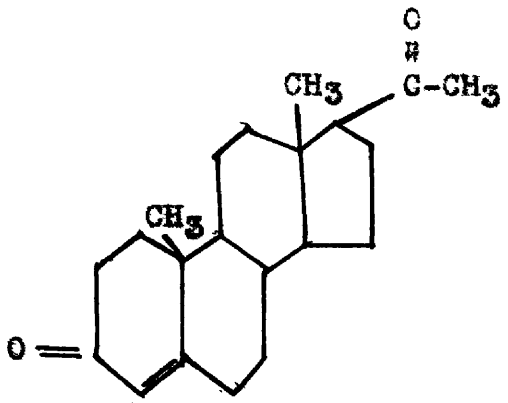
Our results obtained with the castrate heifer give highly significant quantitative information on the hormonal control of the mammary system. The different amounts of ovarian hormones present at different stages in the estrous cycle produce a variable that can be controlled only by ovariectomy. This variability is further magnified by the direct discharge of the animals own hormones into the blood where they are immediately available for use. On the contrary subcutaneous injections are much more slowly available and are consequently more likely to be destroyed before being utilized. Anselmino and Hoffmann (1936) treated normal lactating rats twice daily with 1000 I. U. of estrogen. In 7 litters the young died in 5 to 9 days; in 3 other litters the young survived but were stunted. In ovariectomized animals the same dosage did not inhibit lactation. They concluded that the inhibiting effect was not direct and consequently progestin was proposed as the hormone responsible for inhibition of lactation. Later Anselmino, Harold,

Hoffman and Pencharz (1936) found that 0.5 I. U. doses of progestin suppressed the secretion of milk entirely in young castrate rats when administered at the time of castration and 18 hours later. No inhibition of lactation was observed when 40 to 8000 I. U. doses of estrogen were given to a series of similar animals under the same condition. The report by Folley and Kon (1937) that 1 mg. doses of progesterone injected daily for 15 days in normal lactating rats did not suppress lactation, strongly indicates that this hormone does not inhibit lactation in the normal animal.

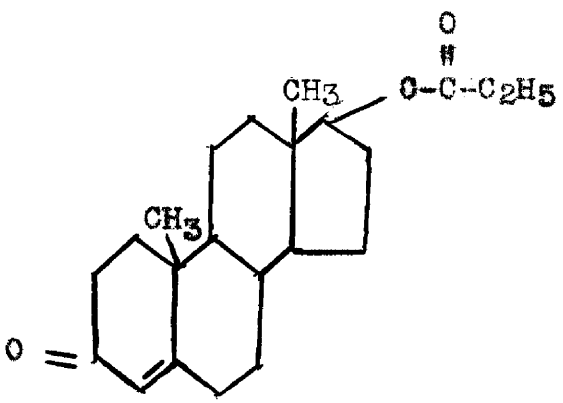
The prolonged period of mammary development in the castrate heifer suggests the operation of a time factor related to the length of the gestation period. This suggestion is in agreement with the observation of Gardner and van Wagenen (1938) in their study of the monkey. Increases in the amounts of the hormones injected in the castrate heifer failed to produce a proportional increase in mammary development.

Selye, McEuen and Collip (1936) reported slight acinar development in the mammary gland of rats injected with testosterone benzoate. Scipiades (1937) found that 5 daily 5 mg. injections of testosterone in rats castrated on the twelfth to seventeenth day of pregnancy prevented abortion. Greene and Burrill (1939) showed that rats castrated after the eleventh day of pregnancy carried living young to term when treated with testosterone propionate. These similarities of biological activity of testosterone together with its

close relation in chemical structure to progesterone suggested



Progesterone



Testosterone propionate

the use of testosterone propionate in combination with estrogen to bring about more complete mammary development than we were able to produce with estrogen alone. Enhancement of rate of mammary gland growth in the castrate heifer occurred when small doses of testosterone propionate were given along with diethylstilbestrol dipropionate. A series of injections in castrate twin kids showed that testosterone propionate is more effective in producing growth of the mammary gland than progesterone when these hormones are injected simultaneously with diethylstilbestrol dipropionate.

The study of mammary glands from which milk is not being removed should throw more light on the suppressing effect of estrogens on the secretory process. Large doses of estrogen in the castrate heifer and in the barren heifer brought about an immediate change of the udder from a turgid to a flaccid condition. These results suggest that at least a part of the inhibitory function may be accomplished by a direct effect upon the mammary tissue.

The large doses of diethylstilbestrol dipropionate preceding the beginning of milking in the castrate heifer appear to be the cause of the slow increase in daily production during the early period of lactation. It should be pointed out, however, that an initial increase in milk volume accompanied small doses of this estrogen administered after a plateau in production had been attained. The decline in daily production which followed a prolonged treatment suggests a cumulative effect of this hormone. Such an effect could be anticipated in light of the observation of Dodds, Goldberg, Lawson and Robinson (1938) that this estrogen induces continuous estrus in the rat for 50 days after a 10 gamma injection. The 53 per cent increase in milk volume 46 days after cessation of estrogen treatment may be interpreted either as a result of stimulation of the secretory process or as a result of additional mammary proliferation. Reece, Bartlett, Hathaway and Davis (1940) found that lactating rats injected with pregnancy urine extract and

estradiol benzoate showed the greatest parenchymal proliferation and the most pronounced suppression of lactation. Mitotic activity of the mammary gland was proposed as an inhibitory mechanism of the secretory process. This interpretation of our results in the castrate heifer would explain the increase in lactation following estrogen treatment as due, at least in part, to additional mammary growth. If on the other hand, the increase is regarded as being due entirely to a direct effect on the secretory process, it may be assumed that a titre of estrogen too high for maximum secretion was maintained during the intervening period between the two points of high production. It must be further assumed that the estrogen titre slowly decreased to a level conducive to stimulation of the secretory process. It should be pointed out that suppression of lactation reported in dairy cows by Folley (1936) was accomplished by 20 mg. doses of estrogen given daily over a period of 5 days. The dosage of estrogen used by Folley was 3 times as large for the 5-day period as the amount used in our experiments with the castrate heifer. We found that daily 0.2 mg. doses of the same estrogen which we used in the castrate heifer suppressed lactation completely in the intact rat, while the same amount of this hormone had little effect on lactation in the castrate animals. In light of these observations the failure of Folley to obtain an increase in milk volume in lactating cows may be attributed to excessive doses. The stimulating effect of diethylstil-

bestrol dipropionate on lactation was reaffirmed in the lactating castrate heifer when 6 alternate daily 10 mg. doses were given. The daily milk yield increased to 15.1 lb. per day during the first 6 days of treatment. With continued injections the daily yield declined to the pre-injection level. After 10 days of production at this level the daily milk volume increased to 16 lb. This double peak in the production curve is a duplication of the results obtained when the first series of estrogen injections was given. The increase in volume represented by the second peak of this curve is regarded as a confirmation of the conclusion that estrogen brings about proliferation of the parenchyma of the lactating mammary gland.

Injections of estrogen alone and injections of estrogen and progesterone together in pre-pubertal castrate kids induced very little mammary gland development when these hormones were given over a period of 114 days. These results are in agreement with our findings in dairy calves, and therefore offer further evidence that an age factor is involved in the development of the mammary gland of juvenile animals.

Even though the extent of mammary gland development in the goats was limited, some observations of the manner and degree of growth induced by the various injections are significant. During the period of injections with 1 mg. doses, pronounced lengthening of the teats occurred in the

animal which was given testosterone propionate alone. It should also be pointed out that no mammary gland enlargement occurred in the kid given testosterone propionate alone while the twin of this animal which was given diethylstilbestrol dipropionate alone showed the most rapid mammary gland enlargement during the period of treatment with 1 mg. doses (Table 4). However, when 2 mg. injections were begun the mammary glands of goats 3 and 4 began to enlarge while no further enlargement occurred in goat 1. After the dosage was reduced to 1.5 mg. the mammary gland of goat 3 continued to enlarge slowly. No further udder enlargement could be detected in goat 4. This observation suggests that the male hormone is a more effective synergist for the mammogenic activity of estrogen than progesterone.

Results obtained by injections of diethylstilbestrol dipropionate in lactating rats have shown that suppression of lactation is much more complete in intact than in castrate animals. Doses of 0.2 mg. brought about more complete suppression or lactation in noncastrate females than 1 mg. doses given to castrates. The results reported by Folley and Kon (1937) showed that 1 mg. doses of progesterone daily, over a period of 15 days, did not inhibit lactation in normal lactating rats. Therefore, it is unlikely that progesterone secreted by the ovaries of the intact animals used in our experiments was sufficient to account for the suppression of lactation that occurred.

Although the inhibitory effects of estrogen on lactation occurred early in the intact rats, the growth rate of the young of these animals exceeded that of the control litters during the second and third days of treatment. In all animals that were given estrogen the initial growth rate of the young was greater than the growth rate of the control litters (Figs. 3 and 4). These results show that estrogen in slightly higher than normal titres is capable of increasing the rate of lactation while the accumulation of an increasingly higher titre suppresses the rate of lactation. These findings reaffirm our observation in the castrate lactating heifer. The first effect of injections of estrogen in this animal was a 17 per cent increase in daily milk volume. Continued injections were followed by a decrease in daily production to the pre-treatment level.

Turner (1939) reported that the rapid enlargement of the mammary glands during late pregnancy is a result of secretion into the alveoli already formed. The rapid increase of estrogen excretion in cattle during late pregnancy (Turner, 1934) suggests that this hormone is being secreted at a high rate when enlargement of the udder is most pronounced. These observations are in agreement with the results of our experiments with the castrate and the barren heifer. In both of these animals an increase in the dosage of estrogen was followed by a rapid enlargement and pronounced turgidity of the udder. Our results are interpreted

as evidence that a medium titre of estrogen stimulates the secretory process of the alveolar cells.

The curve (Fig. 2) which represents the daily milk volume of the lactating castrate heifer shows a striking contrast to the milk production curve during a similar period of lactation in normal cows. In the normal cow a peak of milk production is reached about 5 weeks after parturition. This peak is followed by a slight decline of production during the next several months. A production peak was reached in the castrate heifer 2 months after milking was begun. The 91 per cent increase in yield that followed during the next 4.5 months appears in striking contrast to the gradual decline in the normal cow at the corresponging period of lactation. This 91 per cent increase in milk volume is attributed to parenchymal proliferation of the lactating gland induced by estrogen.

Summary

Acetone dried powder of the anterior lobe of cattle pituitary was administered to three dairy calves. The total dosages given were: 3.4 mg. to a 3.5 month old Jersey, 5 mg. to a 6.5 month old Jersey, and 7.2 mg. to a 6.5 month old Holstein. Slight mammary gland hypertrophy was observed in the 6.5 month old Jersey calf. No apparent udder enlargement occurred in the other 2 calves.

Eighteen mg. of the same pituitary preparation was given to a 34 month old barren Jersey heifer over a period of 17 days. No apparent mammary enlargement occurred. Four large corpora lutea were found by rectal palpation 22 days after the first injection. Subsequent treatment of this animal with alternate daily 5 mg. doses of diethylstilbestrol dipropionate during a period of 20 days was followed by pronounced udder development and induction of lactation. Administration of an equal amount of this hormone after lactation was established had no effect on milk volume or fat content. Lactation continued at a level of 12 pounds per day with a fat content of 7 per cent until the heifer was dried off 8 months after the last injection.

Another experiment with this animal was begun in an attempt to determine the extent of mammary development that could be obtained by injections of diethylstilbestrol dipropionate alone. Alternate daily injections of 5 mg. of this

hormone were begun 130 days after the first lactation period. The animal came in heat, but no mammary gland enlargement occurred. When 10 mg. injections of estrogen were given heat reactions ceased and the udder enlarged rapidly during a period of 10 days. After this short period of rapid enlargement the udder no longer increased in size. When 20 mg. injections of estrogen were given the udder became flaccid. Milking was begun. During the first 25 days of lactation daily milk volume increased from 0.41 lb. to 3.7 lb. At this time alternate daily injections of 5 mg. of estrogen were begun. The daily volume increased to 8.1 lb. during the second 25-day period.

Injection of a 22 month old castrate grade Jersey heifer on each alternate day with 5 to 10 mg. doses of diethylstilbestrol dipropionate over a 2-month period produced considerable udder enlargement. Simultaneous injection of 5 mg. of this estrogen and 1 mg. of testosterone propionate induced marked udder growth. Turgidity of the gland continued with gradually increased doses of these two hormones. Doses exceeding 40 mg. of diethylstilbestrol dipropionate and 10 mg. of testosterone propionate produced little further growth. A flaccid condition of the udder followed injection with 75 mg. doses of the estrogen alone.

After 9 months of treatment milking was begun. The rate of milk secretion increased in 53 days from 0.41 lb. to 8.6 lb.

per day. An average daily volume of 8 lb. was produced during the next 36 days. A total of 110 mg. of diethylstilbestrol dipropionate was then given in alternate daily 10 mg. injections. Milk production increased to 10 lb. during the first 16 days of this treatment and then declined to the pre-injection level by the third day after the last injection. A marked increase in milk volume occurred 36 days after the last injection. Within 18 days the daily production was 53 per cent greater than it had been at any previous period. The milk yield for the next 21 days averaged 13.6 lb. At this time alternate daily injections of 10 mg. of estrogen were begun. By the third day after the first injection the daily production had increased to 16.1 lb. During the next 4 days the milk volume declined to 13.9 lb. Treatment was stopped after the sixth injection. After 10 days of production at the 13.9 lb. level, the daily volume increased during 3 days to 16 lb. Production has remained at this level up to the time of this writing 22 days later.

A group of 6 pre-pubertal kids containing 2 sets of twins were used in the study of 3 problems that arose during the experiments with cattle. These problems are outlined on page 26. Five of the animals were castrated 38 days before injections were begun. Goat 1 was given diethylstilbestrol dipropionate; goat 2, testosterone propionate; goat 3, three parts of the estrogen to 1 part of the androgen; goat 4, three parts of the estrogen to 1 part of progesterone.

Injections were made on each alternate day. Treatment with 1 mg. doses over a period of 46 days induced slight udder growth in goats 1, 3 and 4, and lengthening of the teats in goat 2 (Table 4). The dosage of each hormone was then doubled. The udder of goat 1 became flaccid. The udder of goat 3 increased in size, but no enlargement of the udder of goat 4 could be detected. After 23 days of treatment with 2 mg. doses, 1.5 mg. injections were begun. Since these injections induced no further udder enlargement over a period of 36 days, injections were stopped.

In order to study the effect of diethylstilbestrol dipropionate on lactation, a series of experiments was carried out with 26 multiparous rats. This hormone was given in varied doses to castrate and to intact animals. Injections of 0.2 mg. and 1 mg. were given to separate groups of lactating mothers whose sucklings had been reduced to 6 per litter at parturition. Injections were given from the second to the twentieth day after parturition. The average growth rate of the young was used as the measure of the rate of lactation.

Average growth rate of the young of all groups of treated mothers exceeded that of the controls during the second day of the injection period. After the second day of treatment the young, whose mothers were left intact and were given either large or small doses of the estrogen, gained weight less rapidly than the sucklings of noncastrate

controls. On the contrary the rapid growth rate was continued for 6 days in the young of the castrate mothers which were given 1 mg., and for 12 days in the litters of castrate mothers which were given 0.2 mg. The suppression of lactation was much more pronounced in noncastrate than in castrate animals. Either large or small doses brought about almost complete suppression of lactation in noncastrate animals. Little or no inhibitory effect was observed in 3 of the 4 castrate mothers which were given 0.2 mg.

The pituitary, ovaries, and adrenals of the injected rats showed a marked increase in weight when compared with the glands in the control animals. All treated animals and 3 of the 4 castrate controls lost weight during the period of the experiments, while the intact untreated mothers gained weight.

Conclusions

1. Mammary gland development and induction of 16 lb. of milk per day were brought about by injection of diethylstilbestrol dipropionate and testosterone propionate in a castrate heifer. It was therefore concluded that progestin is not needed for complete development of the mammary gland in cattle.

2. Testosterone propionate when given with diethylstilbestrol dipropionate enhances mammary gland development in castrate cows and goats.

3. Testosterone propionate is a more effective synergist of the mammogenic activity of diethylstilbestrol dipropionate in goats than an equal amount of progesterone.

4. Diethylstilbestrol dipropionate is especially well adapted to the experimental development of the mammary gland. The cumulative effect obtained with this hormone when given over a prolonged period suggests that it is removed from the body at a slower rate than the normally occurring estrogens.

5. The titre of estrogen determines its effect on the mammary gland. A low titre induces proliferation of the parenchyma; a medium titre causes the glandular cells to secrete; a high titre suppresses lactation.

6. Maximum development of the mammary gland occurs when a titre of estrogen is maintained below the level required to induce the secretory activities of the glandular cells.

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Explanation of Figures

Figure 1. A graph showing the curves of lactation, percentage of fat and percentage of solids-not-fat during the early period of lactation a 34 month old barren Jersey heifer injected with 18 gm. of acetone dried anterior lobe of cattle pituitary (A. D. P.), containing 50 R. U. of gonadotropic potency per gm., and 100 mg. of diethylstilbestrol dipropionate (estrogen). Each period of estrogen treatment consisted of 10 alternate daily 5 mg. injections. The curves were stopped at the point in the lactation period where they became level. Four large corpora lutea were found by rectal palpation at the twenty-second day represented in this graph.

Figure 2. A graph showing the lactation curve and fat percentage curve of a 31 month old castrate grade Jersey heifer. Mammary gland development was induced in this animal by injection with 2115 mg. of diethylstilbestrol dipropionate and 236.5 mg. of testosterone during the 9-month period which preceded lactation. The first period of diethylstilbestrol dipropionate (estrogen) treatment represented in this graph consisted of 11 alternate daily 10 mg. injections; the second consisted of 6 alternate daily 10 mg. injections.

Figure 3. A graph showing the growth rate curves of suckling rats whose mothers were left intact. The weights represented in this graph were obtained by dividing the total weight of a given group of young by the total number of sucklings in the group. The weights of litters which received similar treatment and which survived through the 21 days of the experiments were pooled and plotted as a single curve. The weights of those litters in which one or more sucklings died before the end of the 21-day period were plotted on separate curves. These curves were stopped at the point where the first member of a given litter died. All litters were reduced to 6 at parturition.

Figure 4. A graph showing the growth rate curves of suckling rats whose mothers were castrated at parturition. The explanation given for Fig. 3 applies also to the curves plotted in this graph.

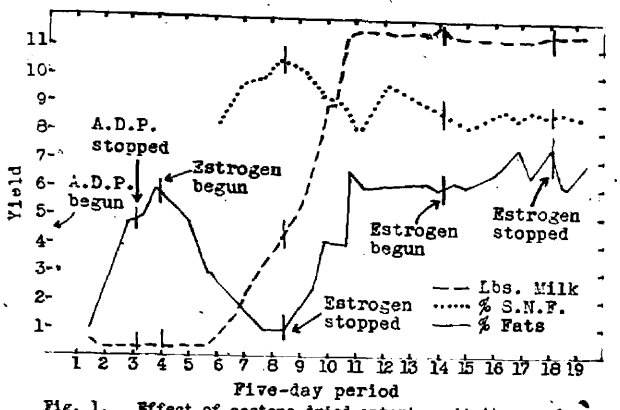

Fig. 1. Effect of acetone dried anterior pituitary and stilbestryl dipropionate on induction of lactation in a 34-month virgin Jersey heifer.

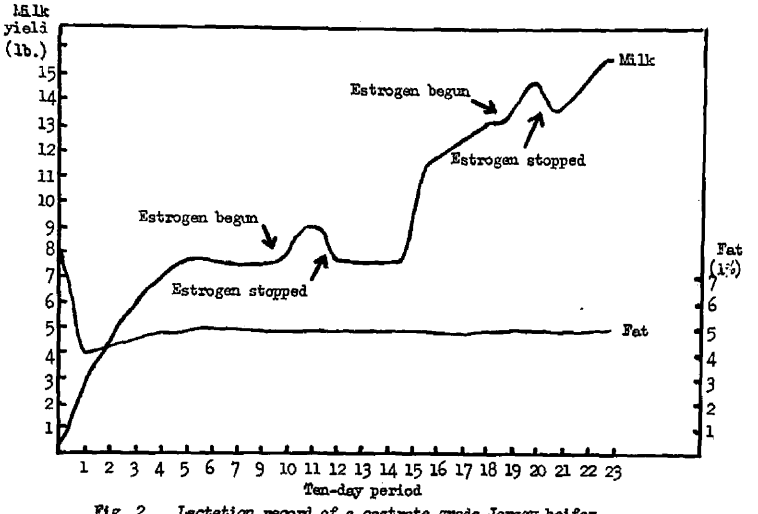

Fig. 2. Lactation record of a castrate grade Jersey heifer injected with diethylstilbestrol dipropionate

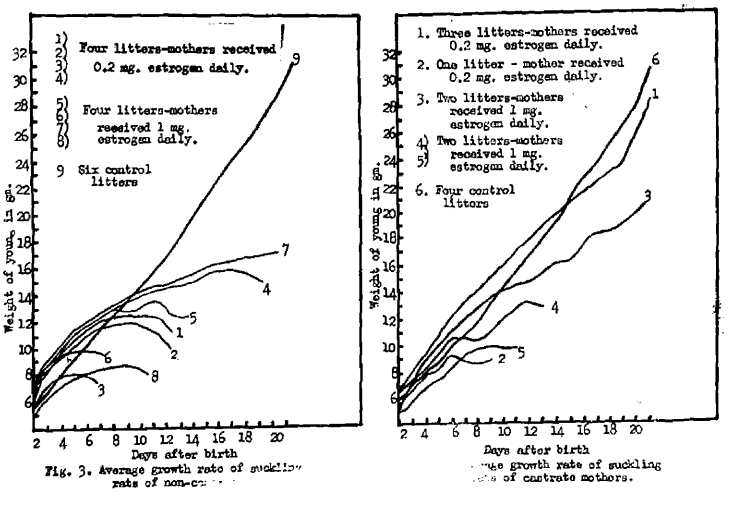

Fig. 3. Average growth rate of suckling rats of non-ca... rats of castrate mothers.

Figure 5. A photograph of the udder of a 3.5 month old Jersey calf before pituitary injection was begun.

Figure 6. A photograph of the udder shown in fig. 5 after the calf was given 2.6 gm. of acetone dried anterior lobe of cattle pituitary, containing 50 R. U. of gonadotropic potency per gm., over a period of 14 days.

Figure 7. A photograph of the udder of a 6.5 month old Jersey calf before pituitary injection was begun.

Figure 8. A photograph of the udder shown in fig. 7 after the calf was given 2.6 gm. of acetone dried anterior lobe of cattle pituitary, containing 50 R. U. of gonadotropic potency per gm., over a period of 14 days. The animal came in heat 8 days before this photograph was taken and from 2 to 15 cc. per day of milky fluid was expressed from the udder during the 16-day period that followed.

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9. A photograph of the udder of a 6.5 month old Holstein calf before pituitary injection was begun.

Figure 10. A photograph of the udder shown in fig. 9 after the calf was given 3.4 gm. of acetone dried anterior lobe of cattle pituitary, containing 50 R. U. of gonadotropic potency per gm., over a period of 14 days.

Figure 9

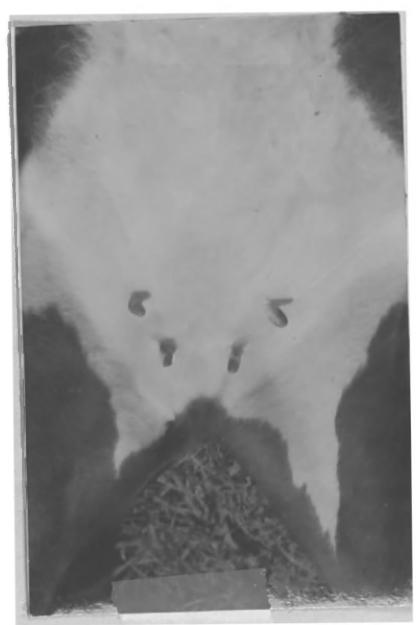

Figure 10

Figure 11. A photograph of a 51 month old barren Jersey heifer which was taken on the thirty-fifth day of the second lactation period. This animal was 34 months old when the experiment which induced the first lactation period was begun. 1/20X.

Figure 12. A photograph of the udder of the animal shown in fig. 11. Injection of 18 gm. of acetone dried anterior lobe of cattle pituitary, containing 50 R. U. of gonadotropic potency per gm., had been given over a period of 17 days. This treatment was followed by 8 alternate daily 5 mg. injections of diethylstilbestrol dipropionate. The animal was 34 months old when injections were begun. 3/10X.

Figure 11

Figure 12

Figure 13. A photograph of the udder shown in figure 12 showing the reduction in size of the udder of the virgin Jersey heifer after milking was stopped. The animal had been milked for 8 months. The photograph was taken prior to the beginning of injections of diethylstilbestrol dipropionate. 3/10X.

Figure 14. A photograph of the udder of the virgin Jersey heifer after 23 alternate daily 5 mg. injections of diethylstilbestrol dipropionate over a period of 44 days and 11 alternate daily 10 mg. injections of diethylstilbestrol dipropionate over a period of 20 days. The udder increased in size most rapidly on the fifteenth day after 10 mg. injections were begun. 3/10X.

Figure 13

Figure 14

Figure 15. A photograph of a 34 month old castrate grade Jersey heifer 7 months after milking was begun. This animal was 10 months old at the time of castration and 22 months old when injections were begun. 1/19X.

Figure 16. A photograph of the udder of the 22 month old castrate grade Jersey heifer before injection of diethylstilbestrol dipropionate was begun. 3/10X.

Figure 17. A photograph of the udder of the castrate heifer 5 months after the first injection of diethylstilbestrol dipropionate was given. Alternate daily injections of 5 to 10 mg. doses of this estrogen were given during the first 67 days of treatment. During the remaining 83-day period the animal was given simultaneous injections of 5 to 30 mg. of diethylstilbestrol dipropionate and 1 to 5 mg. of testosterone propionate in alternate daily doses. The most rapid increase in size of the udder occurred when simultaneous injections of 5 mg. of diethylstilbestrol dipropionate and 1 mg. of testosterone propionate were given. 3/10X.

Figure 15

Figure 16

Figure 17

Figure 18. A photograph of the udder of the castrate grade Jersey heifer after a 9-month period of injections with diethylstilbestrol dipropionate. During the last 7 months of this period testosterone propionate was injected simultaneously with this estrogen. The udder continued to increase in size as the alternate daily dosage was gradually increased to 40 mg. of estrogen and 5 mg. of androgen. When the dosage was increased to 55 mg. of estrogen and 15 mg. of androgen the udder became flaccid. A total of 1520 mg. of diethylstilbestrol dipropionate and 236.5 mg. of testosterone propionate were given during the 9-month period. Milking was begun at the time this photograph was made. 3/10X.

Figure 19. A photograph of the udder of the castrate grade Jersey heifer 2 months after milking was begun. The milk yield was 7.4 lb. per day at the time this photograph was made. 3/10X.

Figure 20. A photograph of the udder of the castrate grade Jersey heifer 5 months after milking was begun. Two months before this photograph was made alternate daily 10 mg. injections of diethylstilbestrol dipropionate were begun. Eleven injections were given over a period of 21 days. This photograph was made 49 days after the last injection at which time the daily yield was 12.1 lb. per day. 3/10X.

Figure 18

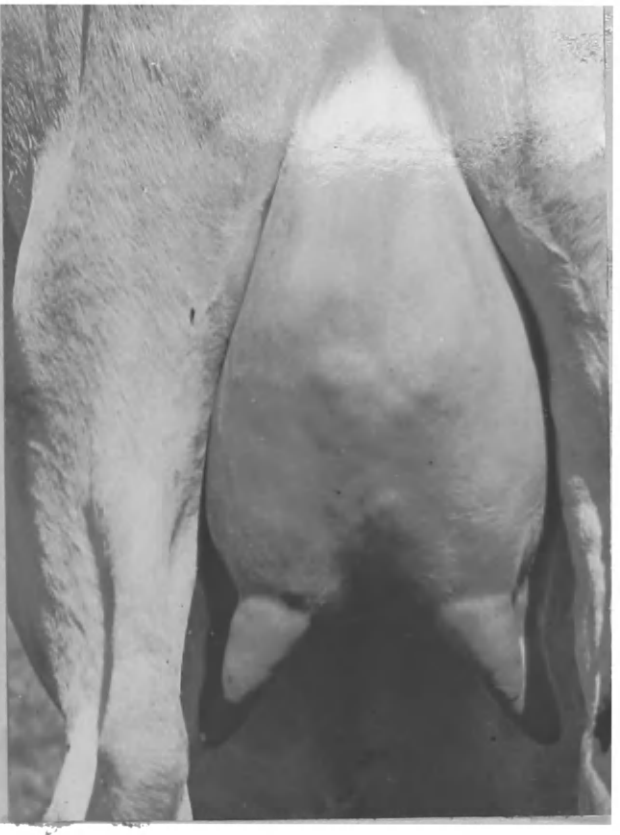

Figure 19

Figure 20

Appendix

The Record of Milk Production of a Castrate Grade Jersey Heifer

Date	Volume in cc.			Per cent fat	Per cent S.N.F.	Remarks
	Morning	Evening	Daily			
Aug.13 1940	350		350	9.1	12.72	Only 1 milking
14	176	128	304	5.8	9.86	
15	112	96	208	8.1	9.57	
16	196		196	5.3	10.11	Only 1 milking
17	300	195	495	4.6	9.42	
18	434	310	744	4.4	9.78	
19	518	400	918	5.1	9.77	
20	386	614	1000	3.7	9.69	4 A.M. and 7 P.M. milkings
21	530	452	982	3.4	9.73	
22	755	500	1255	4.5	9.61	
23	720	666	1386	3.3	9.96	
24	886	544	1430	4.5	10.05	
25	1000	636	1636	4.7	9.44	
26	1020	714	1734	4.6	9.52	
27	990	680	1670	3.9	9.53	Refused new feed
28	1144	570	1714	4.6	9.17	
29	1210	614	1824	5.1	9.07	
30	1340	620	1960	4.8	9.21	
31	1210	840	2050	4.5	9.30	
Sept.1	1230	940	2170	3.7	9.24	
2	1302	950	2252	4.4	9.18	
3	1280	900	2180	4.7	9.29	
4	1380	1000	2380	4.9	9.25	
5	1425	990	2415	4.7	9.24	2 P.M. H_2O withheld
6	1330	1104	2434	4.6	9.52	1b A.M. H_2O given
7	1544	1090	2634	5.2	9.84	
8	1580	1068	2648	4.2	9.14	
9	1480	1200	2680	4.6	9.32	
10	1540	1256	2796	5.5	9.85	
11	1470	1260	2730	4.6	9.16	
12	1600	1140	2740	4.7	9.09	
13	1580	1360	2940	4.0	9.30	
14	1560	1380	2940	4.4	9.38	
15	1720	1150	2870	5.2	9.54	
16	1824	1150	2974	5.3	9.06	
17	1970	1020	2990	5.4	9.15	
18	1820	1250	3070	5.2	9.29	
19	1660	1366	3026	4.9	9.18	
20	1754	1508	3262	4.8	9.16	
21	1910	1275	3185	4.2	9.33	
22	1718	1250	2968	5.4	9.23	
23	2002	1380	3382	5.0	9.19	

Date	Volume in cc.			Per cent fat	Per cent S.N.F.	Remarks
	Morn-ings	Even-ings	Daily			
24	1905	1248	3153	5.1	9.27	Cool rain
25	2148	1338	3486	5.1	9.35	all day
26	2010	1458	3468	4.9	9.38	Cool windy
27	1952	1408	3360	5.0	9.30	night
28	2110	1422	3532	5.0	9.10	
29	2254	1328	3582	4.9	9.13	
30	2122	1522	3644	4.4	9.38	
Oct. 1	2114	1456	3570	5.2	9.49	
2	2200	1458	3658	4.7	8.69	
3	2194	1528	3722	5.3	9.00	
4	2178	1560	3738	5.3	9.11	
5	2182	1502	3684	5.0	8.80	
6	2222	1648	3870	4.6	8.72	
7	2156	1150	3306	5.2	8.99	
8	2824	550	3374	3.5	9.25	A.M. milking
9	2350	1402	3752	5.9	9.68	11:00 and
10	1758	1382	3140	3.4	9.68	photograph
11	2447	1350	3797	7.6	9.37	
12	2320	1325	3645	6.7	9.12	
13	2340	1375	3715	5.5	9.08	
14	2172	1478	3650	5.8	8.76	
15	2162	1455	3617	5.7	8.94	
16	2168	1376	3544	5.4	9.06	Cool night
17	2255	1312	3567	5.7	8.94	P.M. milking
18	2360	1232	3592	5.4	9.43	4:30
19	2131	1380	3511	5.1	9.22	
20	2090	1226	3316	6.2	9.39	
21	2116	1386	3502	5.6	9.10	
22	2040	1452	3492	5.1	9.19	
23	2152	1432	3584	5.5	8.45	
24	2180	1385	3565	5.6	8.57	
25	2140	1372	3512	5.1	8.42	
26	2025	1475	3500	5.7	8.72	
27	2070	1175	3245	5.6	8.52	
28	2010	1380	3390	5.6	9.02	
29	2042	1350	3392	5.1	8.72	
30	2060	1550	3610	5.1	8.97	
31	2025	1450	3475	5.1	8.92	
Nov. 1	2050	1550	3600	5.2	8.96	
2	2117	1650	3767	5.1	8.82	
3	2270	1588	3858	5.1	8.85	
4	2325	1605	3930	5.1	8.62	
5	2171	1676	3847	4.9	8.68	
6	2421	1523	3944	4.8	8.91	
7	2275	1526	3801	5.3	9.04	
8	2202	1510	3712	5.4	8.98	
9	2201	1490	3691	5.4	9.18	10 mg. est.[1]
10	2348	1562	3910	5.1	8.77	

1. Diethylstilbestrol diproprionate

Date	Volume in cc.			Per cent fat	Per cent S.N.F.	Remarks
	Morn-ings	Even-ing	Daily			
11	2312	1585	3897	5.2	9.44	10 mg. est.
12	2375	1637	4012	5.5	9.55	
13	2402	1649	4051	5.6	9.21	10 mg. est.
14	2285	1554	3839	5.7	9.39	
15	2415	1634	4049	5.5	9.45	10 mg. est.
16	2490	1588	4078	5.4	9.38	
17	2569	1452	4021	5.1	9.42	10 mg. est.
18	2533	1761	4294	5.0	9.40	
19	2609	1725	4334	5.6	9.79	10 mg. est.
20	2709	1706	4415	5.0	9.40	
21	2683	1826	4509	4.5	9.15	10 mg. est.
22	2825	1630	4455	5.0	9.50	
23	2810	1755	4565	5.1	9.52	10 mg. est.
24	2894	1692	4586	4.9	9.23	
25	2697	1850	4547	5.1	9.52	10 mg. est.
26	2665	1720	4385	5.3	9.20	
27	2551	1630	4181	5.5	9.65	10 mg. est.
28	2473	1554	4027	5.5	9.55	
29	2412	1562	3974	5.5	9.30	10 mg. est.
30	2375	1425	3800	5.7	9.29	
Dec. 1	2453	1350	3803	5.5	9.55	
2	2100	1432	3532	5.4	9.53	
3	2210	1442	3652	5.7	9.54	
4	2286	1458	3744	5.4	9.58	
5	2130	1458	3588	5.4	9.38	
6	2192	1428	3628	5.5	9.50	
7	2176	1480	3656	5.1	9.47	
8	2228	1375	3603	5.5	9.50	
9	2250	1425	3675	5.5	9.50	
10	2105	1295	3400	5.2	9.39	
11	2095	1375	3470	5.2	9.39	
12	2250	1425	3675	5.2	9.14	Rain
13	2195	1278	3473	5.1	9.37	Rain
14	2225	1511	3736	5.1	9.22	
15	2375	1222	3597	5.1	9.02	
16	2182	1502	3684	5.2	9.04	Cold
17	2431	1375	3806	5.3	9.16	
18	2344	1611	3955	5.0	9.15	
19	2475	1636	4111	5.0	9.29	
20	2432	1300	3732	5.0	9.03	Rain 20-26
21	2840	1190	4030	4.7	9.34	
22	2050	1100	3150	4.8	9.13	
23	2050	1460	3510	5.7	9.04	
24	2408	1372	3780	4.8	9.08	
25	2306	1461	3767	4.5	9.43	
26	2580	1078	3658	4.4	9.03	
27	2240	1495	3735	5.1	9.44	

Date	Volume in cc.			Per cent fat	Per cent S.N.F.	Remarks
	Morning	Evening	Daily			
28	2130	1477	3607	4.8	9.63	
29	2299	1384	3683	5.4	9.23	
30	2116	1304	3420	5.4	9.55	
31	2194	1302	3496	5.6	9.34	
Jan. 1	2064	1550	3614	5.2	8.79	Rain
2	2427	1485	3912	4.9	8.98	
3	2145	1495	3540	4.9	8.98	Rain
4	2154	1247	3401	5.8	9.18	New pasture
5	2500	1360	3860	4.9	9.78	Cold
6	2815	1866	4679	4.8	9.81	
7	2805	1750	4555	4.8	9.86	
8	2955	1900	4855	4.7	9.84	
9	3050	1900	4950	4.5	9.75	
10	3155	2085	5240	4.6	9.40	
11	3155	1955	5110	4.7	9.39	
12	2975	1805	4780	5.0	9.53	Frost
13	3145	2058	5203	5.1	9.52	
14	3225	2220	5445	4.7	9.89	
15	3345	2195	5540	4.4	9.13	
16	3280	2075	5355	4.8	9.60	Rain
17	3377	2200	5577	4.7	9.29	
18	3935	1362	5297	4.9	8.95	Photo 11 A.M.
19	3185	1960	5145	5.0	9.80	Frost
20	4060	1385	5445	4.8	9.54	Photo at
21	3520	2243	5763	4.5	9.53	11 A.M.
22	3425	2240	5665	4.8	9.64	
23	3675	2200	5875	5.3	9.16	
24	3655	2390	6045	4.7	9.49	Rain
25	3550	2355	5905	4.5	9.85	
26	3550	2275	5825	4.6	9.82	
27	3395	2550	5945	5.3	9.76	
28	3705	2410	6115	4.6	9.97	
29	3562	2225	5787	4.6	9.97	
30	3560	2225	5785	4.8	9.96	
31	3560	2225	5785	5.0	10.05	
Feb. 1	3460	2150	5610	5.4	10.03	
2	3735	2245	5980	5.3	10.01	
3	3655	2455	6110	4.6	9.87	Cold
4	3545	2400	5945	4.8	9.96	Frost
5	3490	2500	5990	4.9	9.94	
6	3650	2230	5980	4.8	9.91	Rain all day
7	3650	2020	5670	4.9	9.98	
8	3600	2120	5720	5.1	10.02	Cold
9	3505	2100	5605	5.1	9.92	Heavy frost
10	3425	2365	5790	4.8	9.91	Heavy frost
11	3448	2680	6128	4.7	9.74	
12	3275	2538	5813	4.7	9.79	

Date	Volume in cc.			Per cent fat	Per cent S.N.F.	Remarks
	Morning	Evening	Daily			
13	3625	2430	6055	4.8	9.66	
14	3650	2475	6125	4.6	9.68	Warm
15	3705	2525	6230	4.8	9.66	10 mg. est.[1]
16	3818	2485	6303	4.9	9.68	
17	3970	2645	6615	5.2	9.39	10 mg. est.
18	3990	2810	6800	4.9	9.39	
19	3750	2900	6650	4.9	9.48	10 mg. est.
20	3670	2650	6320	4.6	9.22	
21	3590	2250	5840	5.2	9.39	10 mg. est.
22	3100	2050	5150	5.8	9.76	Cold
23	3025	2160	5185	5.8	9.71	10 mg. est.
24	3525	2365	5890	5.1	9.57	
25	3738	2567	6305	4.4	9.68	10 mg. est.
26	3706	2675	6381	4.8	9.64	
27	3520	2780	6300	5.2	10.29	Swollen external genetalia
28	3650	2695	6345	5.1	10.27	
Mar. 1	3175	3205	6380	5.1	10.07	A.M. milking 5:45
2	3660	2445	6105	5.0	10.25	
3	3692	2765	6457	5.1	9.82	
4	3840	2810	6650	5.2	9.09	
5	3705	2650	6355	5.4	9.88	Cool
6	4020	2824	6844	5.1	9.97	
7	4090	3050	7140	4.7	9.39	
8	4150	2894	7044	5.1	9.52	
9	3850	2090	6340	4.9	9.43	Sent in pasture with a heifer in heat
10	3700	2830	6530	5.3	9.86	
11	3890	2800	6690	5.0	9.90	
12	4030	2870	6900	5.3	10.11	
13	4220	3025	7245	5.5	9.65	
14	4165	2940	7105	5.4	9.63	
15	4142	2935	7077	4.5	9.55	
16	4220	2770	6990	4.7	9.09	
17	4165	2790	6955	4.6	9.12	Cold
18	3884	2970	6854	4.8	9.21	Cold and rain
19	4152	2890	7042	4.7	9.09	Warm
20	4060	3010	7070	4.7	9.14	
21	4200	2800	7000	4.6	9.12	
22	4010	2830	6840	5.1	9.17	
23	3850	3130	6980	5.0	9.05	
24	4050	2970	7050	4.9	9.08	
25	4300	2960	7260	5.1	9.08	
26	4115	3195	7310	5.0	9.05	
27	4460	3075	7535	4.8	9.01	
28	4460	3122	7582	4.8	9.01	

1. Diethylstilbestrol dipropionate

Biography

Sheppard Matthew Walker was born at Perkinston, Mississippi, February 2, 1909. He was graduated from Perkinston High School in 1928 and entered Perkinston Junior College in June, 1928, from which he was graduated in 1930. In September, 1930, he entered the Western Kentucky State Teachers College. From that college he received the B. S. degree in 1932 and the A. M. degree in 1933. During the year 1933-34, he taught General Science at the Glasgow High School, Glasgow, Kentucky. He did post-graduate study at the Western Kentucky State Teachers College in the summer session of 1934. For four years, he taught Biology at Perkinston Junior College (1934-38). In September, 1938, he entered the Louisiana State University where he is now a candidate for the Ph.D. degree.

EXAMINATION AND THESIS REPORT

Candidate: Sheppard M. Walker

Major Field: Physiology

Title of Thesis: The Role of Estrogen in Mammary Gland Development and in Lactation

Approved:

Allan J. Stanley
Major Professor and Chairman

Charles W. Phillips
Dean of the Graduate School

EXAMINING COMMITTEE:

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George H. Mickey

Ellwood A. Pfeiffer

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Date of Examination: